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An introduction to the database of the culture collection of marine microalgae sited at the Oceanography Department of the Spanish Council for Scientific Research, Cádiz (Spain).

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The purpose of this paper is to assert the existence of a database relating the culture collection of marine microalgae sited at the Marine Sciences Institute from Andalusia (Spanish Council for Scientific Research). This collection reports 79 strains, belonging to 10 algal classes, most of them are marine algae, interesting from the aquaculture point of view. The author contributes with the comparative data gathered on the growth characteristics, biomass and energy content of cultured marine microalgae. Cell volume of the algae by distribution range, mean and mode are provided. Gross biochemical composition of the algae, as a percentage of the total protein, carbohydrate and lipid content is also available. To facilitate information about which culture methods (beaker, sterilized plastic, agar) are enjoyed to which strain is also the point here. The database constitutes a reference point for a worldwide scoped collection. It concerns the spread of a scientific object eventually stimulating the exchange of experiences and know-how in this digital domain. It could provide mechanisms for the exchange of open-source software components, and also to establish close contacts with relevant application communities.

Introduction.-

Those researching for model exchange for microalgae species variety between different areas of the world, require the corresponding tools supported by algorithms, software and data. It is recognized that research collections do not give complete information on all the species, and one needs models to relate the volumes of the samples to the number of species and the abundances of them on the one hand and also to the forecasting of the total species on the other. [1] The most productive and fullest conceptual model in this sense is an explicit hypermedia system that can be used to aid navigation and re-orient lost users. [2]

There is also the question of optimal frequency structures in large systems. For the analysis and formalization of the species structure we assume that an indicator of quantity is the number of individuals while the number of species is an indicator of quality [1]. 79 is the total number of marine microalgae strains in this Spanish Council for Scientific Research Collection, belonging to 10 algal classes.

The structure of the presentation is as follows:

In **Section 1** it is discussed the database where the culture collection of marine microalgae electronic table is presented by means of the standard for storing structured text hypertext markup language (HTML). Three distinct stages of hypermedia development has been distinguished: cataloging of the artifacts [ie introduction of the documentation (graphs and summaries of the papers), compilation of the thesaurus providing the classification space, and presentation involving a system controlled form of navigation. Different usages activities have been previewed: directed research oriented activity by the academics, and activities more able to act as tools for curatorial information management.

In **Section 2** the reader can make up his/her mind whether the core group inside the microalgae collection is worth his/her

scientific interests. Either by examining the growth characteristics, biomass, energy content of the microalgae, or their cell volume and gross biochemical composition.

In **Section 3** the filter instrument introduced to supply the information on the culture method is provided.

In **Section 4**, the strategies of scientific research built after this microalgae collection, and acknowledged through the Science Citation Index, are exposed. So, a bibliometric overview diagram of usages of the collection is supplied.

Section 5 discusses five unequally rich information based approaches of the material in the collection. It outlines extensions to the work performed, in particular the modelling of the way the microalgae have been collected.

1. The database of the marine microalgae culture collection of the Oceanography Department of the Spanish Council for Scientific Research, Cadiz (Spain): An hypermedia information system, building and composition.

The Culture collection of Microalgae is assigned to an electronic table written into the simplest implementation between the markup languages, ie hypertext markup language (Marine_microalgae.html). The construction of this table corresponds to the data gathered for authoring and classification by the curator. Thus the structure of the information is defined via intensional relations, offering the capability of providing intelligent assistance in the authoring of the hypermedia material. And the actual occurrences of relationships of relevance to hypermedia nodes are comprised into the extensional information. [2][6]

The scheme of the database matches the electronic table with a thesaurus. A keyword index has been built as a single

comprehensive index and an identified point of access to any data [4].

Related to this driver are the summaries of the reported documents (after the pre-/post- prints facilitated by the authors) where the priorities in research have been claimed for, after the microalgae collection. Throughout their storage life in the database these documents will be interpreted with the curator provided keywords, displayed immediately at the left of the abstract's text, and in the layout of the Video Display Unit.

Another focus of data implements the bibliometric overview which tries to make more attractive the data on the microalgae, from a competitive viewpoint, by reporting some of the uses associated with them after an analysis of the Science Citation Index.

At last a web-designed page retains the memory on previous experiments conducted on the growth characteristics, biomass and energy content of the core group in the microalgae collection.

All the material is designed with Dublin Core metadata ready to be incorporated to an Internet web site.

2. The database: An electronic application supplying with information on growth characteristics, biomass and energy contents. Some proficiencies related with cell volume and gross biochemical composition.

Throughout 20 selected strains, and after seven days (data already published [5]) of growth development, when we move from Cyanophyceae to Bacillariophyceae, the growth characteristics, chlorophyll and energy potentially available data are exposed (into the page [Benchmarking_The_Core_Group.html](#)) both referred to organic matter obtained and to biomass.

The significance of the relations between the numbers of species is inclined to regulate the optimal-nutritional criteria. It

seems that standard indices for species richness, equilibration, and dominance could afford with the applied aspect of aquaculture engineering raised by this approach. [1]

The range of cell concentrations describes a wide interval (from $0,3 \cdot 10^6 \text{ ml}^{-1}$ (*Tetraselmis sp.*) to $118,5 \cdot 10^6 \text{ ml}^{-1}$ (*Synechococcus sp.*)). But when expressed in terms of maximum biomass obtained, the variation is less random (between 139 and $207 \mu\text{g ml}^{-1}$), been the two limiting cases *Synechococcus sp.*, *Tetraselmis Chuui* and *Tetraselmis Suecica* (superior bound) and *N.atomus* and *N.maculata* (inferior bound).

Looking ahead, the values of the growth rates can be expressed between 0,34 and $0,67 \text{ d}^{-1}$. And the exceptions to the bounded area are *Tetraselmis sp* ($1,12 \text{ d}^{-1}$), and *C. gracilis* and *C.calcitrans* ($0,23 \text{ d}^{-1}$ and $0,21 \text{ d}^{-1}$).

When one estimates the Chlorophyll levels, massively influenced by pigment composition and by the metabolism of the strains under the culture conditions, the distribution is splitted up, by means of the regularity of most species, into two differing in cardinality subgroups. When referred after the biomass: the upper one with *Tetraselmis suecica* ($36,7 \text{ mg g}^{-1}$), *Phaeodactylum tricornutum* ($27,8 \text{ mg g}^{-1}$), *Tetraselmis sp* ($24,8 \text{ mg g}^{-1}$), and *Dunaliella salina* ($23,0 \text{ mg g}^{-1}$); and encoded as involving the smallest value: *Nannochloris maculata* ($10,1 \text{ mg g}^{-1}$). And when the index is to be built in terms of $\mu\text{g ml}^{-1}$ the oscillation would be between *Nannochloris maculata* ($0,61 \mu\text{g ml}^{-1}$) and *Synechococcus sp* ($3,91 \mu\text{g ml}^{-1}$).

The energetic value, developed from the chemical composition, varies between *Tetraselmis Suecica* ($26,3 \text{ J mg}^{-1}$) and *Nannochloris maculata* ($55,2 \text{ mg}^{-1}$).

The cell volumes of the algae present an interval of oscillation between $1,4 \mu\text{m}^3$ attached to *Nannochloris maculata*

and 723,0 μm^3 derived from the *Tetraselmis* sp. The means are also between these two algae, ie a range from 3,0 to 419,9 μm^3 respectively. All the distributions have been reported to present asymmetry to the right (positive skewness, the mean is greater than the mode).

The gross biochemical composition of the core group inside the algae collection, as a percentage of the total protein, carbohydrate and lipid content, reveals an accumulation of reserve substances (carbohydrate and lipid content) facing the proteins. And *Synechococcus* resulted in providing maximum protein contents (minimum of lipids), *Nannochloris maculata* affording with the minimum of proteins, and *Chaetoceros calcitrans* presenting the maximum of lipids.

3. The key instrument introduced to supply the information on the stock culture method is provided.

Mastery of techniques lay at the center of microalgae pursuit. Tools like media challenge the algologist's ability to grow, isolate, and observe strains in the laboratory, since the environment provided in the laboratory induces changes into the morphological traits used for identification proposed. And the eventual compression of the data in accordance with the species structure from optimal variety to anomalous state can demonstrate to the users (possessors) of the collection that an adequate representation and expression is available from the layout of the electronic table presented by html means.

Agar is a seaweed product, with the advantage of remaining firm at body temperature, the preferred growing temperature for most algae. It is symbolized with an A. The culture is performed in a tube with quarterly renewal (see [Marine_microalgae.html](#)).

All subsequent stock cultures are maintained either in 100 ml beaker, what is marked up with M in the electronic table; or in a

6 ml tube of sterilized plastic, expressed with a T. (Both procedures with weekly renewal.)

The filtered and sterilized sea water is enriched with Guillard's f/2 medium.

4. Bibliometric overview diagram of usages of the collection

Under this heading it is proposed to evaluate the context of the citations produced towards the publication:

Lubián,LM^a; Yúfera,M. (1989) Culture collection of microalgae of the Marine Sciences Institute of Andalusia (CSIC). In: Acuicultura intermareal. 1989. M. Yúfera (Ed.). Inst.Cien.Mar.Andalucía, Cádiz. pp.69-78.

This expertise is available visualizing the results of a short citation study inside the Science Citation Index. Additional data has been derived from the presence of this collection inside the Cambridge Scientific Abstracts databases on aquatic science. The remain analysis approaches those publications from the Oceanography Dpt. (CSIC. Cádiz) not included into SCI, but resting on the collection for the management of the strains.

Following closely the distribution of the amounts of references under each entry of the thesaurus, we can presume that the bibliometric appraisal of the presence of the Marine Microalgae Culture Collection, at Cádiz CSIC Station, into the Cambridge Scientific Abstracts (ASFA, Aquatic Sciences and Fisheries Information System), maintains similar properties. Interesting enough is the position of temporal monopoly on spanish total production in what concerns scientific journal articles, for the period considered 1997-2000. (What could be contrasted with the data on Ph.D. produced by the labo doctoral students, no more than 6 over the 60 amounting for the total spanish production).

In particular, set against the seven microalgae bibliographies were Cádiz-CSIC labo. was detected, the only spanish references available were those identified with the staff of the Oceanographic Dpt. And this despite the eventual uninvolved with productions (like turbot) known to need as microalgae diet a particular one included in the collection (as *Tetraselmis chuii*).

Intimately connected with the rise of *Nannochloropsis gaditana* Lubián as a new species 4 over the 5 contributions on this microalgae, in ASFA, come from CSIC-Cádiz Marine Microalgae Collection. Equally, the percentual rates of the collection in ASFA are: 14% of the production on *Rhodomonas baltica*, 11% on *Tetraselmis chuii*, 11% on *Nannochloropsis oculata*, 100% on *Nannochloris maculata*, 100% on *Nannochloris atomus*, and 4% on *Chaetoceros gracilis*.

More data extracted from the databases report on the presence of the sequence of the 18S rRNA gene of *Nannochloropsis gaditana* Lubián, in GenBank and in the DNA Databank of Japan (DDBJ).

5. Additional retrieval questions to facilitate navigation through the database

The following questions for developing benchmarked perspectives between the species presented in the collection (and under the three nominal regions in which they could be splitted up, ie species that are not numerous, intermediate ones, and ones predominant in numbers) are proposed:

[1] Is it culturing the prerequisite for identification?, ie Does the phenotype expressed in pure culture necessarily reflect the phenotype(s) expressed in the natural setting?

[2] Do the major phylogenetic assemblages serve specific community functions linked to carbon cycling?, or Is it the

mineralization of organic carbon a way to approach the collection?

[3] Has the question on sulphate reduction as an (obligately) anaerobic process make sense in ICMAN's collection of marine microalgae?, ie Which is the identity of the microalgae populations associated with aerobic sulfate reduction?

[4] Which way were collected the microalgae (by coring and subsampling)?, At which increments?, How were transported the samples?, In water?, Under which conditions of temperature?, Was constancy of temperature obtained?, Which kind of material (aluminium?) is employed to cool the samples?

[5] Could be determined the marine microalgae that may function as nutritional generalists?, Which is the range of substrates they oxidize?

When asking:

[1] Is it culturing the prerequisite for identification?, ie Does the phenotype expressed in pure culture necessarily reflect the phenotype(s) expressed in the natural setting?

It could be retrieved from the database that:

In rapidly identifying marine microalgae taxon-specific attributes of plants may be of use. Limited to the division or class level the identification of algal phylogenetic groups rely on pigment "signatures". Work on [Ultrastructure and pigments of some planktonic chlorophyceae and Eustigmatophyceae of similar morphology Nannochloris, Nannochloropsis, Algae, taxonomic aspects](#) . has been performed after the culture collection of microalgae here at Puerto Real. Mass mortality of farmed bluefin tuna (an area where CSIC station in Cadiz has been notorious in the past) has derived into experiments on the different light

adaptation phenotypes after long-term culturing under similar physiological conditions.

[2] Do the major phylogenetic assemblages serve specific community functions linked to carbon cycling?, or Is it the mineralization of organic carbon a way to approach the collection?

From the database, and under the titles: Effects of high irradiance and temperature on photosynthesis and photoinhibition in *Nannochloropsis gaditana* Lubián (Eustigmatophyceae), relationships between bio-optical characteristics and photoinhibition of phytoplankton, and A comparative study of acid and alkaline phosphatase activities in several strains of *Nannochloris* (Chlorophyceae) and *Nannochloropsis* (Eustigmatophyceae) are some of the pertinent retrievable items.

In fact, specific for microalgal phylogenetic groups chlorophylls and carotenoids indicate the activity of the Xanthophyll cycle acting as a photoprotection system. The carbon fixation rates remarked when researching on the Xanthophyll cycling as optimizer of the biosynthesis of light-harvesting Xanthophylls under fluctuating light conditions, have been studied after strains from the collection.

[3] Has the question on sulfate reduction as an (obligately) anaerobic process make sense in ICMAN's collection of marine microalgae?, ie Which is the identity of the microalgae populations associated with aerobic sulfate reduction?

It has been observed that *Haematococcus pluvialis* is one of the favored organisms for biotechnological production of Keracarotenoids, antioxidative compounds, but without relationships with the strain present in the collection.

[4] Which way were collected the microalgae (by coring and subsampling)?, At which increments?, How were transported the samples?, In water?, Under which conditions of temperature?, Was constancy of temperature obtained?, Which kind of material (aluminium?) is employed to cool the samples?

According to the database no more than the last question could be answered.

Works on Effects of slow and rapid warming on the cryopreservation of marine microalgae and Effects of culture age on cryopreservation of marine microalga, have been retrieved. After what, the development of marine microalgae cryostorage might contribute to improve hatcheries efficiency by assuring the permanent and immediate availability of high-quality and stable stock cultures. The algae were the following: *Tetraselmis chuii*, *Nannochloris atomus*, *Nannochloropsis gaditana* (showing the best tolerances to biological freezing, with mean viabilities of 97.9%, 80.5% and 61.6% respectively), and *Rhodomonas baltica* and *Isochrysis galbana* (with the lowest viability). The material employed to cool the samples was a Cryoson BV-25 biological freezer, which was filled with a platinum sensor for temperature and two copper thermocouples (connecting the sample with the CPU, and recording the environmental temperature in the freezing chamber).

Searching for producing more information on the cryopreservation of the marine microalgae of scientific and commercial interest a viability index for cryopreserve marine microalgae employing different cooling rates, with or without cryoprotective compounds, has been conducted. *Tetraselmis chuii*, *Nannochloropsis gaditana* and *Nannochloris atomus* Butcher could be cryopresrved without cryoprotectant. *Isochrysis galbana* gave the poorest results. *Rhodomonas baltica* was very poorly cryopreserved at the higher salinity (as associated with freshwater environments). Between the diatoms, *Chaetoceros gracilis* achieved the higher post-throw viability.

[5] Could be determined the marine microalgae that may function as nutritional generalists?, Which is the range of substrates they oxidize?

When examining the differences in the mode of inorganic carbon utilization it has been shown (in: Comparative study of dissolved inorganic carbon utilization and photosynthetic responses in *Nannochloris* (Chlorophyceae) and *Nannochloropsis* (Eustigmatophyceae) species), after strains from the collection, that *Nannochloropsis* species are able to transport bicarbonate across the plasmalemma. *Nannochloris* species was affected by the inhibitor acetazolamide.

A further question on an effective means of increasing microalgal populations within confined environments could be answered after microalgae cultures from the the collection. The population growth of microalgae in monospecific culture and their related bacteria has been found the subject of a document in the database.

Conclusion.-

I end with a final generalization:

When the establishment of the new algal class, *Eustigmatophyceae*, was stated, some botanists wished to explain the differences between *Nannochloris* and *Nannochloropsis* microalgae genres. The historical record of 1982, (Lazaroa, 4: 287-293.), when *Nannochloropsis gaditana* Lubián showed to be a new species of the genus *Nannochloropsis*, confines to the semantics of a theory the development of a Microalgae culture collection.

Data have retained a powerful hold on problems using the theoretical tools already at hand. And those whose main concern is the application of scientific findings to ecotoxicology and aquaculture problems have approach the boundaries of their criterias by allocating resources for the creation of a particular database.

The qualitative indicator suggested by the 49 different species cultured in the collection has been attained with the expansion in knowledge provided by the use of data for comparisons. Through achieving systematic comparative studies between microalgae strains the collection has grown, and this is the background of the database.

The database building can shed light on the conditions which determine the uses of the strains, the pattern of species diversity, and the meaning of the activities entrenching the laboratory system and the microalgae collection.

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